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Antifungal Activity of Components of Essential Oils

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Antifungal activities were examined and compared for some 40 kinds of aliphatic and aromatic aldehydes, alcohols, phenolic compounds, ether compounds and hydrocarbons from essential oils and for some related compounds, using seven fungi.

Considerable studies on antimicrobial activities of essential oils and their components have been reported.[1~12] However, systematic investigations using many kinds of components of essential oils have yet been very limited.

Thus, in the present studies, we examined the antifungal activity of about 40 kinds of compounds from essential oils and several related compounds, using 7 fungi. Furthermore, the relationship between antifungal activity and molecular orbital energy was discussed for carbonyl compounds.

MATERIALS AND METHODS

Reagents. Caryophyllene and perillalcohol were kindly supplied by Mr. Takashi Yasuda of Takasago Perfumery Co., Ltd. All of the other compounds employed here were of commercial source.

Fungi. The fungi employed for assays of antifungal activity were listed in Table I. These fungi, after isolated from clinical specimens, had been maintained on Sabouraud dextrose agar slants at room temperature at the Research Institute for Chemobiodynamics, Chiba University.

Assays of antifungal activity. The assay was carried out at 27°C on a 2% glucose Sabouraud agar slant containing one compound to be tested. Before use, each compound to be tested was dissolved in ethyl ether for sterilization, then added into sterile culture medium at a specified concentration. A small amount of ethyl ether added with each compound tested did not affect the growth of any of the fungi employed. Two percent glucose Sabouraud agar without any addendum was used as a control medium. Ten to fifteen-day old culture of each fungus on a 2% glucose Sabouraud agar slant was used as an inoculum onto the control and test media throughout the present investigation. Antifungal activity of a compound was estimated based on the duration of inhibition of fungal growth under the presence of the compound. Whether fungal growth occurred or not was determined macroscopically.

Molecular orbital calculations. Molecular orbital calculations were performed by the Hückel molecular orbital method with π-electron approximation, using parameters following Yonezawa *et al.*[13] The hyperconjugation model was applied to a methyl group.

RESULTS AND DISCUSSION

Antifungal activity of aliphatic aldehydes and ketones

Results are shown in Table I. Cinnamaldehyde was the highest in antifungal activity among the aliphatic aldehydes examined. The antifungal effects of perillaldehyde and citral were somewhat weaker than that of cinnamaldehyde but still fairly potent. In contrast, the antifungal effects of citronellal, octanal, nonanal and decanal were all very poor.

TABLE I. ANTIFUNGAL ACTIVITY OF ALIPHATIC ALDEHYDES AND KETONES FROM ESSENTIAL OILS

Compound	Duration of growth inhibition (day)						
	Fungus						
	Blastomyces dermatitidis	*Histoplasma capsulatum*	*Trichophyton rubrum* (H)	*Fonsecaea pedrosoi* (T)	*Aspergillus nidulans*	*Penicillium frequentans*	*Penicillium cyclopium*
Cinnamaldehyde							
0.25 mM	3	> 20	0	0	0	0	0
0.33 mM	> 20	> 20	3	0	1	0	0
0.50 mM	> 20	> 20	> 20	0	> 20	0	0
0.66 mM	> 20	> 20	> 20	2	> 20	1	1
1.00 mM	> 20	> 20	> 20	> 20	> 20	> 20	> 20
Perillaldehyde							
0.25 mM	2	3	0	0	0	0	0
0.50 mM	> 20	12	7	1	0	1	1
1.00 mM	> 20	> 20	> 20	3	2	4	4
2.00 mM	> 20	> 20	> 20	9	> 20	14	> 20
Citral							
0.25 mM	1	2	0	0	0	0	0
0.50 mM	6	> 20	10	0	0	0	0
1.00 mM	> 20	> 20	> 20	1	3	2	1
2.00 mM	> 20	> 20	> 20	2	> 20	3	6
Citronellal							
1.00 mM	1	0	0	0	0	0	0
2.00 mM	4	4	2	0	0	0	0
Octanal							
1.00 mM	0	0	0	0	0	0	0
2.00 mM	0	2	1	0	0	1	0
Nonanal							
1.00 mM	0	1	1	0	0	0	0
2.00 mM	0	> 20	6	0	0	0	0
Decanal							
1.00 mM	0	1	1	0	0	0	0
2.00 mM	0	> 20	11	0	0	0	0
D(+)-Carvone							
1.00 mM	1	5	1	0	0	0	0
2.00 mM	7	> 20	3	1	0	0	0
D-Camphor							
2.00 mM	0	0	0	0	0	0	0

Thus it is apparent that as for the aliphatic aldehydes, those which have one or more double bonds conjugated to their carbonyl group are much higher in antifungal activity than those which have not. This is in line with the results previously reported by Kurita *et al.*[2]

The antifungal activity of carvone, an α,β-unsaturated ketone, was considerably lower than those of perillaldehyde and citral, α,β-unsaturated aldehydes, but was much higher than that of camphor, an α,β-saturated ketone.

Antifungal activity of aromatic aldehydes. Results are presented in Table II. The anti-

TABLE II. ANTIFUNGAL ACTIVITY OF ALIPHATIC ALDEHYDES

Compound	Duration of growth inhibition (day)						
	Fungus						
	Blastomyces dermatitidis	*Histoplasma capsulatum*	*Trichophyton rubrum* (H)	*Fonsecaea pedrosoi* (T)	*Aspergillus nidulans*	*Penicillium frequentans*	*Penicillium cyclopium*
Benzaldehyde							
1.0 mM	3	0	0	0	0	0	0
2.0 mM	> 20	0	0	0	0	0	0
***p*-Methylbenzaldehyde**							
0.5 mM	1	0	0	0	0	0	0
1.0 mM	6	0	1	1	1	0	0
2.0 mM	> 20	2	2	3	2	1	1
Cuminaldehyde							
0.5 mM	> 20	11	2	0	0	0	0
1.0 mM	> 20	19	6	2	1	2	2
2.0 mM	> 20	> 20	> 20	4	6	5	6
***p*-Anisaldehyde**							
1.0 mM	> 20	0	0	0	0	0	0
2.0 mM	> 20	19	1	0	0	0	0
Vanillin							
1.0 mM	0	0	0	0	0	0	0
2.0 mM	1	> 20	1	1	0	0	0
Furfural							
1.0 mM	0	0	0	0	0	0	0
2.0 mM	1	1	2	0	2	1	0

fungal effect of benzaldehyde was very weak or nil except on *Blastomyces dermatitidis*. Cuminaldehyde (*p*-isopropylbenzaldehyde) was the highest in antifungal activity among the aromatic aldehydes examined, and its antifungal effect was fairly potent. The antifungal activity of *p*-methylbenzaldehyde was intermediate between those of benzaldehyde and cuminaldehyde. The antifungal effects of *p*-anisaldehyde (4-methoxybenzaldehyde) and vanillin (4-hydroxy-3-methoxybenzaldehyde) were very poor except on one or two fungi. Furfural, although very toxic to animals and the human being, was very weak in antifungal effect on any of the fungi employed.

Antifungal activity of alcohols. Results of antifungal effects of primary alcohols are given in Table III. The antifungal effect of cinnamylalcohol was very weak except on *B. dermatitidis* and *Histoplasma capsulatum*.

However, the antifungal effects of the other primary alcohols were considerably potent.

Results of the effects of secondary and tertiary alcohols are shown in Table IV. The results presented in Tables III and IV appear to indicate that as for alcohols from essential oils, primary alcohols are in general considerably higher in antifungal activity than secondary and tertiary alcohols.

It is of interest to note that each α,β-saturated carbonyl compound listed in Table I was much lower in antifungal activity than a corresponding alcohol compound (citronellal *vs* citronellol; octanal *vs* 1-octanol; nonanal *vs.* 1-nonanol; decanal *vs.* 1-decanol; D-camphor *vs.* borneol) (Tables I, III and IV). The reason why a hydroxyl group is much more effective than a carbonyl one for these compounds to

TABLE III. ANTIFUNGAL ACTIVITY OF PRIMARY ALCOHOLS FROM ESSENTIAL OILS

Compound	Duration of growth inhibition (day)						
	Fungus						
	Blastomyces dermatitidis	*Histoplasma capsulatum*	*Trichophyton rubrum* (H)	*Fonsecaea pedrosoi* (T)	*Aspergillus nidulans*	*Penicillium frequentans*	*Penicillium cyclopium*
Cinnamylalcohol							
0.5 mM	4	0	0	0	0	0	0
1.0 mM	>20	>20	0	0	0	0	0
2.0 mM	>20	>20	1	1	0	0	1
Perillalcohol							
0.5 mM	2	0	0	0	0	0	0
1.0 mM	19	>20	1	1	0	1	1
2.0 mM	>20	>20	>20	10	2	18	>20
Geraniol							
0.5 mM	11	14	1	0	1	0	1
1.0 mM	>20	>20	>20	1	2	3	4
2.0 mM	>20	>20	>20	15	>20	>20	>20
Citronellol							
0.5 mM	3	14	2	0	0	0	0
1.0 mM	>20	>20	>20	1	4	5	7
2.0 mM	>20	>20	>20	>20	>20	>20	>20
1-Octanol							
1.0 mM	0	3	0	1	0	2	1
2.0 mM	16	>20	7	4	3	>20	6
1-Nonanol							
0.5 mM	3	4	0	0	1	1	1
1.0 mM	12	>20	11	1	2	3	4
2.0 mM	>20	>20	>20	7	4	5	6
1-Decanol							
0.5 mM	10	>20	2	0	1	1	1
1.0 mM	>20	>20	>20	2	2	2	3
2.0 mM	>20	>20	>20	4	4	7	9

exhibit the antifungal effect still remains unclear.

Antifungal activity of phenolic compounds. Results are given in Table V.

p-Cresol (4-methylphenol) was about two times higher than phenol in antifungal activity; *p*-ethylphenol was about three times higher than *p*-cresol; and *p-n*-propylphenol was about two times higher than *p*-ethylphenol. The antifungal activity of thymol (2-isopropyl-5-methylphenol) was approximately equal to that of *p-n*-propylphenol.

The antifungal activity of eugenol (4-allylguaiacol) was eight to ten times higher than that of guaiacol (*o*-methoxyphenol), and three to four times higher than that of creosol (4-methylguaiacol). The activity of isoeugenol (4-propenylguaiacol) appeared slightly higher than that of eugenol.

As is obvious from the molecular structure, addition of alkyl group(s) to benzene ring of phenol or of guaiacol remarkably enhanced the antifungal activity, and the activity of these phenolic compounds appeared to be depend-

Table IV. Antifungal Activity of Secondary and Tertiary Alcohols from Essential Oils

Compound	Duration of growth inhibition (day)						
	Fungus						
	Blastomyces dermatitidis	*Histoplasma capsulatum*	*Trichophyton rubrum* (H)	*Fonsecaea pedrosoi* (T)	*Aspergillus nidulans*	*Penicillium frequentans*	*Penicillium cyclopium*
(Secondary alcohol)							
2-Octanol							
1.0 mM	0	0	0	0	0	0	0
2.0 mM	0	1	1	0	1	1	0
L-Menthol							
1.0 mM	4	6	6	0	0	0	1
2.0 mM	11	>20	7	0	0	0	4
Borneol							
1.0 mM	0	0	0	0	0	0	0
2.0 mM	7	12	4	0	0	0	1
(Tertiary alcohol)							
Linalool							
1.0 mM	0	0	0	0	0	0	0
2.0 mM	2	14	0	0	0	0	1

ing upon the size of the added alkyl group, *i.e.*, the larger the size of the alkyl group, the higher was the antifungal activity.

This enhancing effect of alkyl groups was also observed in the series of benzaldehyde, *p*-methylbenzaldehyde and cuminaldehyde (Table II).

Since alkyl groups are hydrophobic, these results appear to indicate that more than a certain degree of hydrophobicity is also required for phenolic compounds and aromatic aldehydes to exhibit a potent antifungal effect.

Antifungal activity of ether compounds and hydrocarbons. Results are given in Table VI. Among ether compounds examined, 1,8-cineole and safrole were very weak in antifungal effect on any of the fungi. In contrast to them, the effects of methyl eugenol and methylisoeugenol were potent and comparable to those of eugenol and isoeugenol. These results appear to indicate that the free hydroxyl group of eugenol and isoeugenol is not indispensable to their potent antifungal activity.

All of 7 hydrocarbons examined here were almost ineffective in inhibiting the growth of any of the fungi employed at a concentration of as high as 2 mM

Consideration on the relationship between the antifungal activity of the carbonyl compounds and their molecular orbital energy

There have been a considerable number of reports concerning the relationship between a biological activity of compounds and their molecular orbital energy.[14]

Recently, we[12] found that the antifungal activity of aliphatic aldehydes (cinnamaldehyde, perillaldehyde, citral and citronellal) is closely correlated with the calculated energy value of their lowest empty molecular orbitals, and these aldehydes except citronellal are capable of forming a charge transfer complex with tryptophan, a good electron donor.[15,16] Based on these results, we suggested a possibility that the antifungal activities of cinnamaldehyde, perillaldehyde and citral are, at least in part, due to their ability to form charge transfer complexes with electron donors of a fungus cell.

In the present investigation, more extensive

TABLE V. ANTIFUNGAL ACTIVITY OF PHENOLIC COMPOUNDS

Compound	Duration of growth inhibition (day)						
	Fungus						
	Blastomyces dermatitidis	*Histoplasma capsulatum*	*Trichophyton rubrum* (H)	*Fonsecaea pedrosoi* (T)	*Aspergillus nidulans*	*Penicillium frequentans*	*Penicillium cyclopium*
Phenol							
2.0 mM	0	0	0	0	0	0	0
3.0 mM	0	4	0	0	0	1	0
5.0 mM	1	15	0	1	0	6	3
8.0 mM	>20	>20	8	8	2	>20	>20
10.0 mM	>20	>20	>20	>20	2	>20	>20
p-Cresol							
2.0 mM	0	0	0	0	0	0	0
3.0 mM	2	>20	1	2	0	3	4
4.0 mM	>20	>20	>20	5	1	>20	>20
5.0 mM	>20	>20	>20	>20	>20	>20	>20
p-Ethylphenol							
0.5 mM	0	0	0	0	0	0	0
1.0 mM	>20	>20	2	1	0	1	4
2.0 mM	>20	>20	>20	>20	6	>20	>20
p-*n*-Propylphenol							
0.5 mM	>20	>20	>20	2	0	3	>20
1.0 mM	>20	>20	>20	>20	>20	>20	>20
Thymol							
0.5 mM	>20	>20	>20	0	0	1	4
1.0 mM	>20	>20	>20	5	6	>20	19
2.0 mM	>20	>20	>20	>20	>20	>20	>20
Guaiacol							
4.0 mM	0	0	0	0	0	0	0
6.0 mM	2	4	1	1	0	1	1
8.0 mM	7	>20	2	2	1	2	2
10.0 mM	>20	>20	>20	10	1	>20	>20
Creosol							
1.0 mM	0	0	0	0	0	0	0
2.0 mM	3	>20	0	1	0	0	1
4.0 mM	>20	>20	>20	11	1	5	10
Eugenol							
0.5 mM	2	3	2	0	0	0	0
1.0 mM	>20	>20	>20	2	1	1	1
2.0 mM	>20	>20	>20	>20	>20	>20	>20
Isoeugenol							
0.5 mM	>20	>20	18	0	1	1	4
1.0 mM	>20	>20	>20	5	4	11	>20
2.0 mM	>20	>20	>20	>20	>20	>20	>20

studies were carried out, using 7 kinds of aldehydes including those mentioned above and 5 kinds of aromatic aldehydes.

Table VII gives calculated energy values of

TABLE VI. ANTIFUNGAL ACTIVITY OF ETHER COMPOUNDS AND HYDROCARBONS FROM ESSENTIAL OILS

Compound	Duration of growth inhibition (day)						
	Fungus						
	Blastomyces dermatitidis	*Histoplasma capsulatum*	*Trichophyton ruhrum* (H)	*Fonsecaea pedrosoi* (T)	*Aspergillus nidulans*	*Penicillium frequentans*	*Penicillium cyclopium*
(Ether compound)							
1,8-Cineole							
2.0 mM	0	0	0	0	0	0	0
Anethole							
2.0 mM	0	2	1	0	0	0	0
Safrole							
1.0 mM	0	1	1	0	0	0	0
2.0 mM	1	3	2	1	0	1	2
Methyl Eugenol							
1.0 mM	>20	>20	15	2	1	1	3
2.0 mM	>20	>20	>20	8	10	6	8
Methyl Isoeugenol							
1.0 mM	>20	>20	>20	3	3	5	6
2.0 mM	>20	>20	>20	>20	>20	>20	>20
(Hydrocarbon)							
D-Limonene							
2.0 mM	0	0	0	0	0	0	0
α-Pinene							
2.0 mM	0	0	0	0	0	0	0
β-Pinene							
2.0 mM	0	0	0	0	0	0	0
Camphene							
2.0 mM	0	0	0	0	0	0	0
β-Myrcene							
2.0 mM	0	0	0	0	0	0	0
β-Caryophyllene							
2.0 mM	0	0	0	0	0	0	0
p-Cymene							
2.0 mM	0	0	0	0	0	0	0

the lowest empty and highest occupied molecular orbitals of these compounds. The energy of the lowest empty molecular orbital (LEMO) is taken as a relative measure of electron-accepting ability (electron affinity), and that of the highest occupied molecular orbital (HOMO) is taken as a relative measure of electron-donating ability (ionization potential).[17] The lower the energy level of the LEMO, the greater will be the electron-accepting properties. The higher the energy level of the HOMO, the greater will be the electron-donating properties.

As shown in Table I, the antifungal activities of the aliphatic aldehydes were in the following order: cinnamaldehyde > (perillaldehyde, citral)> (citronellal, octanal, nonanal, decanal). From these results and the energy values of molecular orbitals of these compounds, it is obvious that as far as these aliphatic aldehydes are concerned, the lower the energy level of the LEMO, the higher is the antifungal activity. On the other hand, there was no definite correlation between the anti-

TABLE VII. ENERGY OF THE LOWEST EMPTY AND HIGHEST OCCUPIED MOLECULAR ORBITALS OF THE ALDEHYDES

Energy of an orbital is $E=\alpha+\lambda\beta$, α and β being negative quantities. The energy values shown in the table are values of λ in units of β.

Compound	Energy of	
	LEMO*	HOMO**
(Aliphatic aldehyde)		
Cinnamaldehyde	−0.282	0.809
Perillaldehyde	−0.385	0.940
Citral***	−0.389	0.890
Citronellal***	−0.732	0.890
Octaldehyde	−0.732	2.732
n-Nonylaldehyde	−0.732	2.732
n-Decylaldehyde	−0.732	2.732
(Aromatic aldehyde)		
Benzaldehyde	−0.431	1.000
Cuminaldehyde	−0.431	1.000
p-Anisaldehyde	−0.487	0.431
Vanillin	−0.498	0.275
Furfural	−0.363	0.777

* LEMO=the lowest empty molecular orbital.
** HOMO=the highest occupied molecular orbital.
*** Molecular orbital calculations were performed for terpinolene type of citral and citronellal, respectively.

fungal activity of these aldehydes and their HOMO energy level. This is in line with the results previously reported by us.[12]

In contrast to the aliphatic aldehydes, it was difficult to find a definite relationship between the antifungal activity of the aromatic aldehydes and their calculated LEMO or HOMO energy level. Other factors, like hydrophobicity and cell-membrane permeability, may also be required for aromatic aldehydes to exhibit a potent antifungal effect.

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